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Point-of-use detection of ascorbic acid using a spectrometric smartphone-based system

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Abstract

A rapid and portable analytical methodology has been developed for ascorbic acid (Vitamin C) quantification from aqueous samples using a spectrometric smartphone-based system for the first time. The method employs point-of-use approaches both for sample preparation and sample measurement, demonstrating the capability for mobile quality control of pharmaceutical and food products. Our approach utilizes an oxidation-reduction reaction between ascorbic acid and methylene blue, followed by a dispersive liquid-liquid microextraction (DLLME) to extract the aqueous-phase methylene blue into organic media. Then, a back-extraction procedure is employed to transfer the methylene blue to aqueous media, followed by analysis of the sample's absorption spectrum using the spectrometric smartphone-based system. The DLLME and back-extraction procedures are optimized by use of a two-step multivariate optimization strategy. Finally, vitamin C supplements and orange juice are used as real-world samples to assess the applicability of the smartphone-based method, which is successfully compared with the standard laboratory-based approach.

Keywords: Point-of-use detection. Smartphone-based system. Multivariate optimization. Vitamin C quantification. Orange juice. Vitamin C supplements.

1. Introduction

The United States is the second largest global producer of oranges, comprising about 11% of the international market (Reddy *et al.* 2013). The principal vitamin available in oranges is ascorbic acid (AA), one of the 13 essential vitamins for human nutrition, which, in addition to being a required enzymatic cofactor for processes including collagen and neurotransmitter creation, is also reported to serve as a natural antioxidant, reducing the oxidative damage caused by free radicals (Nimse & Pal 2015). Thus, AA has gained increased significance in several areas of analytical chemistry including both pharmaceutical and nutrition applications. As such, the loss of nutritional value that can result from the processing and storage of orange products at room temperature due to a number of deteriorative reactions is an important consideration for growers, transporters, and retailers. This degradation may occur due to reactions between some constituents present in juice products or by the reaction between some constituents of the juice when exposed to oxygen in the air (Johnston & Bowling 2002). Therefore, reliable information about its content in foodstuffs is a concern to both consumers and quality control agencies, and it is essential to develop a simple, fast, and portable method for its determination in routine analysis. The need for new methods for the determination of AA is increasing due to the variety of samples for analysis and the influence of different matrices in an expanding variety of products (Spínola *et al.* 2014).

According to the literature, the development of new methods for the determination of AA is required for routine analysis and several approaches have been reported for determination of AA in food products and vitamin C supplements, including ultraperformance liquid chromatography (UPLC) and high-performance liquid chromatography (HPLC) coupled to a PDA detector (Klimczak & Gliszczyńska-Świgło 2015; Mazurek & Jamroz 2015), voltammetry, amperometry (Jadav *et al.* 2018), UV-Vis spectrometry (Zang *et al.* 2017) and fluorescence (Krishnan *et al.* 2016). In addition, these methods can be very attractive in terms of their limit of detection (LOD), which can extend to concentrations less than 0.1 μg mL^{-1}. However, they present considerable drawbacks, such as the dependence on sophisticated, costly, and bulky instruments, time-consuming analysis, and the need for expensive reagents in high volumes.

Special attention must be paid for ascorbic acid determination using infrared spectrometry techniques. Fourier transform infrared attenuated total reflectance (FT-IR-ATR) (Yulia *et al.* 2014), FT-Raman(Yang & Irudayaraj 2002) and near (NIR) and mid (MIR) infrared spectrometry (Oliveira-Folador *et al.* 2018; Yang & Irudayaraj 2002) are affordable compact instruments that do not need sample preparation. However, there are some drawbacks associated to IR spectrometry. Data interpretation can be complex due to the wavelength dependence of the penetration depth, causing spectral distortion (Fabian *et al.* 2005).

During the past decade, research in analytical chemistry has been focused on the development of a diversity of paper-based sensors (Gong & Sinton 2017), biosensors (Inan *et al.* 2017) and other devices, and analysis with smartphone-based readout platforms. The technical capabilities of smartphones can be readily adapted to enable real-time, low-cost, point-of-use chemical analysis. In fact, smartphone-based platforms have already been demonstrated for several chemical and biological analysis modalities that include pathogen detection (Chen *et al.* 2017; Ganguli *et al.* 2017), cancer biomarker analysis (Hosu *et al.* 2017), characterization of drugs (Yu *et al.* 2016), food safety (Zeinhom *et al.* 2018), and healthcare applications (Kanchi *et al.* 2018). Specifically, the extensive availability of smartphone cameras and image processing techniques allow for low-cost spectrophotometric and colorimetric analysis for a wide variety of sensing applications. In addition, smartphones offer an exceptional platform with a high degree of market penetration and near-ubiquitous data connectivity around the world that can support cloud-based smart service systems for aggregation and analysis of data from a community of users. To validate these capabilities, several manuscripts concerning colorimetric smartphone detection have been published describing uses including the quantification of phosphorus in soil (Moonrungsee *et al.* 2015), potassium in drinking water (García *et al.* 2011), dyes in water (Özdemir *et al.* 2017), free chlorine in water (Wan *et al.* 2016), salivary alcohol concentration (Jung *et al.* 2015), and portable enzyme-linked immunosorbent assays (ELISA) (Long *et al.* 2014), among others.

Though there has been much progress developing point-of-care devices, the use of a spectrometric smartphone-based system for AA quantification has not been previously reported. There are only a few examples of portable optical systems for AA quantification that have been demonstrated (Coutinho *et al.* 2017; Hong & Chang 2014; MeloFerreira *et al.* 2015), but they are all colorimetric in nature, and frequently have significant challenges in differentiating between AA and commonly interfering substances present in commercial products expected to contain AA, such as orange juice (Table S3).

A general disadvantage for miniaturized and portable systems (e.g., smartphone-based systems) is the lack of sensitivity, and therefore high limits of detection in comparison with those obtained with conventional benchtop instruments. One common way to increase sensitivity and to decrease limits of detection is analyte separation and enrichment, which effectively increases the concentration of an analyte. One applicable approach that is compatible with use of miniaturized sample preparation is liquid-phase microextraction (LPME). LPME simultaneously provides simplicity, ease of use, minimal sample and solvent consumption, and minimized generation of chemical byproducts (Moreda-Piñeiro & Moreda-Piñeiro 2015).

Several LPME techniques have been developed, with dispersive liquid–liquid microextraction (DLLME) being the most commonly used (Campillo *et al.* 2017). DLLME is a microextraction methodology based on the dispersion of a few microliters of an organic extractant solvent in an aqueous sample, which can be accomplished in several ways, for example with a disperser solvent. After extraction, phase separation is performed, and the analyte in the organic phase can be analyzed. Since many fine droplets of organic solvent are dispersed throughout the aqueous solution, the very large interfacial area makes the DLLME process both efficient and quick.

The analytical methodology reported here employs the DLLME and back-extraction using methylene blue (MB) for the quantification of AA by a spectrometric smartphone-based system for the first time. The resulting method uses strategies compatible with a point-of-use paradigm for both sample preparation and detection. The method has been optimized by a multivariate optimization approach, and its ability to accurately measure AA concentrations in real samples (i.e., Vitamin C supplement and orange juice) has been established. Finally, the results of the proposed method were compared with those obtained by a conventional titrimetric method.

2. Experimental

2.1.Multimode smartphone biosensing

The optical design, fabrication and main features of a recently-developed smartphone-integrated handheld detection instrument have been previously introduced (Long *et al.* 2017; Scherr *et al.* 2017). Fig. 1A shows the design of this smartphone-coupled spectrophotometric detection system. Although the Transmission, Reflection, Intensity (TRI)-Analyzer is capable of performing three different spectroscopic classes of measurements, we will use the system solely for optical transmission analysis to measure the absorption spectrum of the test sample when it is held in a custom cartridge that integrates a linear series of fluid compartments. Briefly, the illumination fiber (100 μm diameter, multimode) is placed directly in front of the flash LED of the smartphone to direct white light through the test sample. After passing through the sample, the light is back-reflected by a mirror, so light passes through the test sample twice. The sensing fiber (100 μm diameter, multimode) collects the reflected light as it exits the test sample on its second pass. The light emerging from the distal end of the sensing optical fiber is collimated by an achromatic lens (focal length = 19 mm) and then focused in the non-spectral dimension with a cylindrical lens (focal length = 9 mm) before passing through a 1200 lines/mm transmission diffraction grating (Edmund Optics 49-578) held within the cradle body directly over the opening of the rear-facing camera. The proximal ends of the sensing and illumination fibers are gathered together in a bifurcated configuration, so they are directly adjacent and held within a glass capillary tube that is mounted in a slot within the cradle body. Fig. 1B and 1C show absorption spectra and raw red-green-blue (RGB) images of measured spectra from AA concentrations of 0 to 80 μg mL^{-1}, respectively.

2.2.Reagents and samples

For the DLLME assay: A standard stock solution of ascorbic acid (AA) (1,000 μg mL^{-1}) and a standard stock solution of methylene blue (MB) (1,000 μg mL^{-1}) were prepared by dissolving AA standard (VWR International, Radnor, PA, USA) and MB standard (Sigma-Aldrich, St. Louis, MO, USA) in distilled, deionized water, respectively. Working solutions were prepared by dilution of each stock standard solution. Chloroform (Sigma-Aldrich) was used as extractant solvent and acetonitrile (Fisher Scientific, Fair Lawn, NY, USA) was used as a disperser solvent. Diluted hydrochloric acid solution, prepared from a Suprapur 30% (w/w) HCl solution (Fisher Scientific), was used for pH adjustment. Reactive grade NaCl was purchased from Sigma-Aldrich. H_2SO_4 (98%) and PBS (phosphate buffered saline) solutions were purchased from Fisher Scientific and Lonza Inc. (Walkersville, MD, USA), respectively. For the interference study, citric acid was purchased from Fisher Scientific and glucose from Merck (Darmstadt, Germany), while Malic and Lactic acids were purchased from Sigma-Aldrich.

For the reference method: A stock solution with 200 µg mL^{-1} of 2,6-dichloroindophenol (DCPI) (Sigma-Aldrich) was prepared and was standardized by titration with freshly prepared AA solution. The AA solution was prepared by appropriate dilution of AA standard to a concentration of 1,000 µg mL^{-1} by diluting with metaphosphoric acid-acetic acid solution. The later solution was prepared by adding HPO_3 85% (w/w) (Sigma-Aldrich) and acetic acid (Fisher Scientific) up to 4% (w/v) and 8% (w/v), respectively. Distilled deionized water (18.3 MΩ cm) from a Millipore water purification system (Millipore Corporation, Bedford, MA, USA) was used throughout this work. Both DCPI and AA solutions were stored in the dark at 4ºC until used.

Performance characterization on vitamin tablets and orange juice: Commercial vitamin C tablets (Spring Valley™) were purchased in a supermarket. One vitamin C tablet containing 500 mg of AA was diluted to 1,000 µg mL^{-1} in metaphosphoric acid-acetic acid solution. Natural orange juice was obtained by squeezing fresh oranges and then filtering it. Finally, the orange juice sample was diluted ten times to be within in the assay's calculated working range. Both types of samples were stored in the dark at 4ªC until used.

2.2.1. Multivariate optimization

The reduction-oxidation reaction between MB and AA has been well known for several decades. The MB, a blue dye, is reduced in the presence of AA to a colorless compound. The MB extraction can be influenced by several experimental factors that were optimized by a multivariate approach. The main experimental factors affecting the AA determination using DLLME and back-extraction procedures (i.e., extractant solvent volume, disperser solvent volume, sample pH, salt addition (NaCl), back-extraction solvent and back-extraction volume) were optimized using a multivariate analysis consisting of two steps: (i) a Plackett-Burman design (screening) followed by (ii) a circumscribed central composite design (CCCD) (optimization). This study was carried out using the multimode smartphone biosensing TRI-Analyzer platform (Section 2.1.) and a model sample containing 20 μg L^{-1} of AA and 150 μg mL^{-1} of MB to optimize the assay procedure. In both designs, twelve experiments were randomly performed to nullify the effect of extraneous factors.

Plackett–Burman design is a fractional factorial design that ignores interaction between factors and therefore saves both resources and time as main effects can be calculated with a reduced number of experiments. The Plackett–Burman design is advantageous at the beginning of the optimization when many factors are initially considered but finally only a few of them show substantive effects (Montgomery 2009).

Circumscribed Central Composite Design (CCCD) combines a two-level full factorial design (2^k) with $2k$ star points, where k represents the number of factors being optimized, and one point is located at the center of the experimental region. In order to ensure the rotatability of the model, star points were set at $\alpha\pm1.414$ whereas the central point was repeated three times to provide an orthogonal design (Montgomery 2009).

The peak measurement intensity at a wavelength of λ=610 nm, where MB shows maximum absorbance, was used as the response function in both Plackett-Burman design and CCCD. After DLLME and back-extraction procedures, the MB concentration can be very high and MB dimers can form, shifting the wavelength of the maximum absorbance peak from λ=665 nm (MB monomer) to λ=610 nm (MB dimer) (Douissa *et al.* 2013).

2.3. DLLME and back-extraction procedures

Under optimum conditions, 150 µg mL^{-1} of MB, 20 µg mL^{-1} of AA and 10% (w/v) of NaCl solutions were added in a 15 mL test tube, the pH was corrected to 8 and the final volume was adjusted to 10 mL. pH measurements were performed with a pH meter (model Orion 3 Star, Thermo Scientific, Waltham, MA, USA). After a reaction time of 10 minutes, a mixture of 100 µL of extractant solvent (i.e., chloroform) and 300 µL of disperser solvent (acetonitrile) was added using a syringe. A cloudy solution immediately formed and the phase separation was allowed to proceed for one minute. Chloroform was chosen over non-toxic solutions (e.g., octanol) as it allows the phase separation to occur without centrifugation, allowing for a truly portable sample preparation. Afterwards, the aqueous phase was removed and the organic phase was retrieved with a pipette and deposited in an Eppendorf tube of 0.5 mL. For the back-extraction, 19.4 µL of 1 M H_2SO_4 was added to the organic phase and the mixture was inverted by hand for one minute. Since direct measurements of the organic phase were not compatible with the acrylic-based plastic cartridges used for the TRI-Analyzer, back-extraction was necessary. After back-extraction, 8 µL of the enriched, acidic, aqueous supernatant was analyzed by the smartphone-based system. From beginning to end, the sample preparation lasts less than 5 minutes and the overall procedure is graphically described in Fig. 2.

2.4.Titrimetric method

The most widely accepted method of analysis for vitamin C determination in vitamin C supplements and juices is the 2,6-dichloroindophenol (DCPI) titrimetric method (AOAC Method 967.21) (AOAC-International 2005). This method is recommended for the analysis of L-ascorbic acid in vitamin C supplements, beverages and juices for nutritional labeling purposes (AOAC-International 1993). Due to its simplicity, this method is routinely applied worldwide to other food matrices. Although, the deficiencies of the method are well-known, the procedure provides reliable measures for L-ascorbic acid provided that the food does not contain appreciable quantities of both reducing substances and L-dehydroascorbic acid (Eitenmiller & Landen Jr. 1995). Ascorbic acid reduces the indicator dye to a colorless solution. At the endpoint, the excess of unreduced dye is a rose-pink color in acid solution. Therefore, the DCPI works as an auto-indicator.

2.5.Data processing

A multivariate optimization strategy was performed to determine the optimum conditions for the microextraction method. Statgraphics statistical computer package "Statgraphics Centurion XVI" (Warrenton, VA, USA) was used to construct the experimental design matrices and evaluate the results. Image-analysis software is developed with computational software (Matlab, MathWorks, Natick, MA, USA) to process spectral data acquired by the smartphone.

3. Results and discussion

3.1. DLLME and back-extraction optimization

3.1.1. Screening step

Table S1 (supplementary data) shows the experimental factors and levels considered in the Plackett–Burman design. The results obtained from the Plackett–Burman design were evaluated using an ANOVA test and they were visualized with the Pareto chart shown in Fig. S1 (supplementary data). The length of each bar was proportional to the influence of the corresponding factor and the effects that exceed the reference vertical line can be considered significant with 95% confidence level. White bars indicate positive effects (i.e., favorable DLLME conditions at higher values of that factor), while negative effects (*i.e.*, favorable conditions at lower values of the variables) are indicated by black bars.

It can be observed from Fig. S1 (supplementary data) that extractant solvent volume and back-extraction volume were statistically significant factors, with >95% confidence level, showing positive and negative effects, respectively. The positive effect of the extractant solvent volume agrees with the fact that, in general, greater extractant solvent volume involves a greater amount of analyte extracted and therefore increases the measurable response. For back-extraction volume, the negative effect is easily explained by the fact that a smaller volume of acid used results in a higher concentration of the analyte in the final solution. On the other hand, sample pH, back-extraction solvent disperser volume, back-extraction time and NaCl addition were shown to be insignificant in the DLLME and back-extraction procedures. Therefore, the sample pH and the NaCl addition were fixed at their higher level for subsequent extractions (i.e., sample pH: 8; NaCl addition: 10% /w/v)), and the rest of the factors were fixed at their lower level (i.e., back-extraction solvent: 1M H_2SO_4; disperser volume: 300 µL; back-extraction time: 1min). Only extractant solvent volume and back-extraction volume were considered for optimization in the following study.

3.1.2. Optimization step

Table S2 (supplementary data) shows the low and high levels, the central and star points of the factors considered in the optimization step. The response surface obtained by use of the CCCD is shown in Fig. 3. The surface graph shows a pronounced rise in the response as extractant solvent volume increases and the back-extraction volume decreases. Optimal values for extractant and back-extraction volumes were observed to be higher than 100 µL and lower than 19.4 µL, respectively. For ease-of-use, back-extraction volume was not investigated at volumes lower than 19.4 µL. Similarly, as chloroform is an environmentally toxic solvent, volumes of greater than 100 µL were deemed inappropriate for use with this assay. For these practical considerations, volumes were set at these respective limits.

In summary, the results obtained from the optimization process led to the following experimental conditions: Extractant solvent volume: 100 µL; back-extraction volume: 19,4 µL; sample pH: 8; NaCl addition: 10% /w/v); back-extraction solvent: H_2SO_4 (1M); disperser volume: 300 µL; back-extraction time: 1 min. It should be borne in mind that even although hazardous solvent (i.e., only 100 µL) is employed in the present analytical methodology, its consumption is extremely low and it takes advantage of the fact that it does not need centrifugation to separate the organic phase and therefore it can be performed in virtually any location and field environment.

3.2.Analytical figures of merit

Analytical figures of merit of the combination of DLLME and the smartphone-based measurement platform were evaluated to assess the analytical capability of this procedure for the determination of AA in aqueous samples. Under optimized conditions, the working range was established between 20 and 80 µg mL^{-1}. The calibration curve was constructed using five concentration levels, evaluated in triplicate. The resulting calibration curve results in a high level of linearity with a correlation coefficient (r) of

0.998 (N=5). The sensitivity of the instrumental measurements estimated by the slope of the calibration curve was (-12.7±0.5) mL μg^{-1}. The repeatability of the proposed method, expressed as coefficient of variation (CV), was evaluated by five consecutive analyses of 40 μg mL^{-1} AA resulting in a CV value of 8%. The limit of detection (LOD) and the limit of quantification (LOQ) were estimated by using the mean signal of the blank (n = three replicates) plus three or ten times its standard deviation, respectively. The LOD was found to be 5 μg mL^{-1} (27 μM), and the LOQ was 16 μg mL^{-1} (89 μM). To our knowledge, few analytical methods for AA quantification using portable optical systems have been published, and none of them are capable of providing spectrally-resolved analysis (Table S3 in supplementary data) (Coutinho, *et al.* 2017; Hong & Chang 2014; MeloFerreira, *et al.* 2015). It should be noted that, as a result of the spectrometric capability of the device, our observed LOQ values of AA in this work are significantly lower than those obtained using colorimetric approaches using either a smartphone (LOQ 100 μg mL^{-1}) (Hong & Chang 2014) or a desktop scanner (LOQ 276 μmol L^{-1}) (MeloFerreira, *et al.* 2015). Two studies demonstrate LOQ values either slightly better (via portable transmittance) (MeloFerreira, *et al.* 2015) or better (via smartphone image analysis) (Coutinho, *et al.* 2017), though we believe that in each case our demonstrated methodology provides significant improvements. In the former, the paper-based sensor is neither reusable nor stable for periods greater than three weeks, even when stored under refrigeration and in the darkness, resulting in significant challenges for practical point-of-use testing. In the latter, the effect of interfering species was not evaluated, even though an enzymatic oxidation for quantification is likely to have possible signal interference in a complex media such as orange juice. Furthermore, both of these works used different statistical approaches to calculate the LOQ without experimental validation of those calculations, with LOQ values calculated as less than ten times the lowest assayed AA concentration. In our work, we demonstrate a LOQ within the range of assayed AA concentrations both with and without interfering species, providing a substantively more realistic look at real-world usage cases.

3.3. Interference study

In order to assess the possible analytical application of the smartphone-based method, the effect of concomitant species on the determination of ascorbic acid in representative real-world samples was studied by analyzing aqueous solutions containing 40 µg mL^{-1} of ascorbic acid and various excess amount of the common foreign species present in orange juices (e.g., citric, lactic, and malic acids, and glucose) (Scherer *et al.* 2012). Even though other vitamins (e.g., provitamin A carotenoid, vitamins E and D), other organic acids (e.g., tartaric and malic acids) and other amino acids (e.g., arginine, lysine, tyrosine, etc.) could potentially interfere with sample measurements, the concentrations of some of these species in orange juice are significantly lower than the concentration of AA (Barba *et al.* 2011; Gómez-Ariza *et al.* 2005; Restuccia *et al.* 2017; Sánchez-Moreno *et al.* 2003). Moreover, the vitamin C supplement analyzed does not contain rye, soy, yeast, preservatives and lactose.

In this study, a substance was considered not to interfere if the relative error of the true and found concentrations was less than 10%. The results are given in Table 1. They show that citric, lactic and malic acids have the same maximum tolerated ratio (i.e., 30) and that glucose did not substantially interfere with AA quantification (maximum tolerated ration of 45). In addition, the interferent/analyte ratios typically encountered in orange juices and vitamin C supplements are normally lower than the ratios investigated in this interference study.

3.4. Real samples analysis

Tables 2 and 3 show the results obtained for the determination of AA in a vitamin C supplement and natural orange juice, respectively. The results were compared with those obtained by the reference method (AOAC Method 967.21) (AOAC-International 2005) of analysis of AA and a high level of agreement was found (i.e., recovery values of 104 and 99 % of vitamin C supplement and natural orange juice sample, respectively). In addition, natural orange juice was spiked at 16.5 μg mL^{-1} of AA (Table 3). The spiked concentration was close to the observed limit of quantification. According to these results, there was not a significant difference between the concentrations added and that found in the natural orange juice sample, resulting in a recovery value of 98±4 %. The results obtained clearly demonstrate that the spectrometric smartphone-based system has an enormous potential for commercial applications. The analytical instrument used provides important advantages such as portability, fast, reliable AA quantification, and a platform that can be readily adapted to a number of analytes, some of which have already been demonstrated (Gallegos *et al.* 2013; Long, *et al.* 2014; Long, *et al.* 2017; Yu *et al.* 2014).

4. Conclusion

In this work, a smartphone-based absorption spectrometer has been successfully combined with DLLME and back-extraction procedures for the determination of AA in aqueous media. The multivariate optimization approach employed in this research permitted the determination of optimal conditions for the main experimental factors involved in the DLLME and back-extraction procedures in an efficient way. Under optimized conditions, a working range between 20 and 80 μg mL^{-1} was obtained with a correlation coefficient of 0.998 for five calibration points. The LOD and LOQ obtained were 5 and 16 μg mL^{-1}, respectively. The repeatability of the proposed method was evaluated at 40 μg mL^{-1} and a coefficient of variation of 8% was obtained in both cases.

The performance of the proposed methodology was evaluated in vitamin C supplement and natural orange juice and the results demonstrate the ability of the method to determine AA in samples representative of those used in real-world quality control applications. Recoveries values between 104% and 99% were obtained.

The promising analytical methodology proposed here presents a new advance in the development of portable and economical systems available to any laboratory. We envision the adoption of such approaches throughout the chain of suppliers, manufacturers, distributors, packagers, and consumers to easily and quantitatively verify the content of nutrients in food and pharmaceutical products.

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Conflicts of Interest

The authors declare no conflict of interest.

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FIGURES

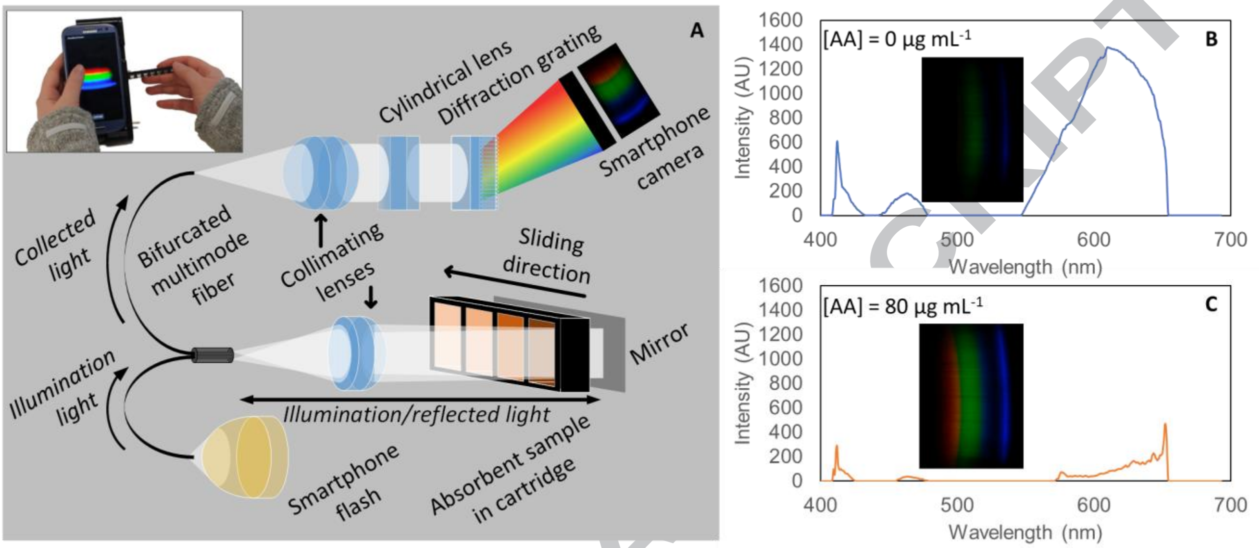

Fig. 1. Schematic illustration of spectral TRI-analyzer and absorption spectra. A: Schematic of internal layout of optical components. Inset: image of final device and resultant absorption spectrum is shown. B and C: Absorption spectra and raw RGB image data (insets) for 0 and 80 µg mL^{-1} of AA, respectively.

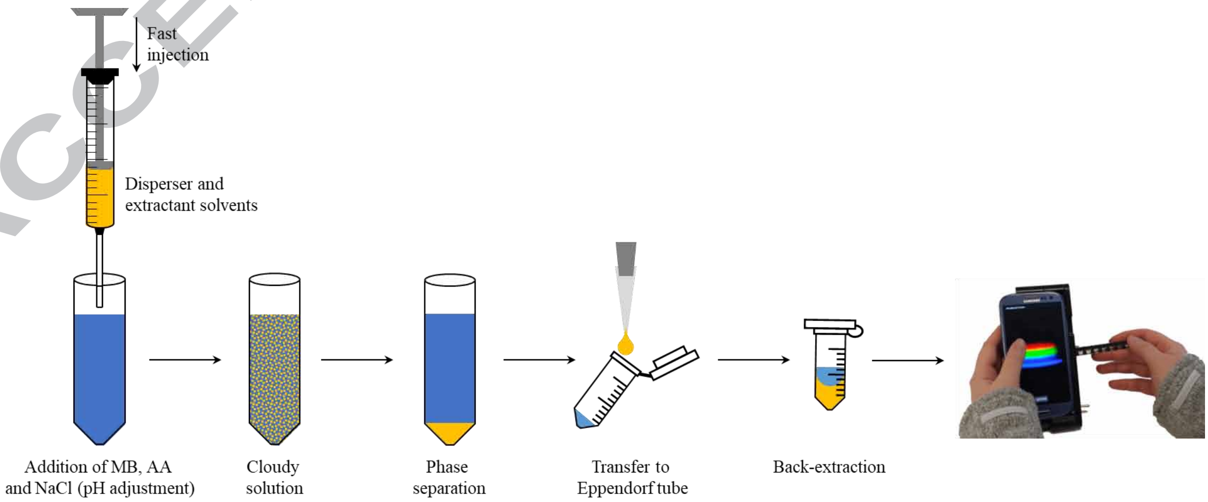

Fig. 2. Scheme of the analytical procedure for AA quantification.

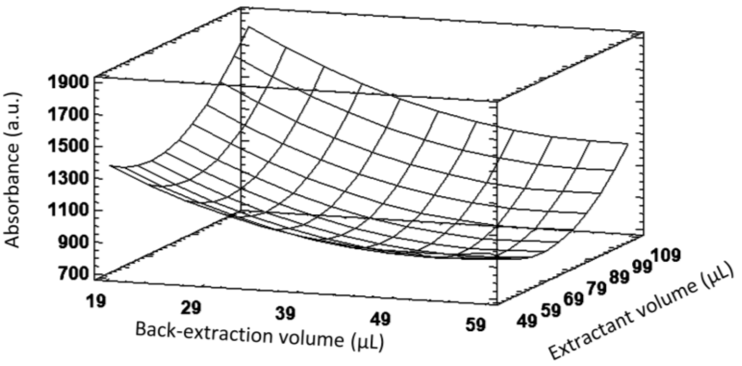

Fig. 3: Response surface of CCCD

TABLES

Table 1: Effect of interferences on determination of 40 µg mL^{-1} of AA. The numbers in parentheses are the relative errors (i.e., RE) of true (i.e., C_t) and found (i.e., C_f) concentrations. [a]

Species	AA concentration (µg mL^{-1})	Found concentration (µg mL^{-1})				
		Interferent/AA ratio				
		0	15	30	45	60
Citric acid	40.4	39.3±0.9 (-3%)	41.4±0.9 (2%)	37.7±0.9 (-8%)	52.8±0.9 (31%)	79.0±1.2 (96%)
Glucose	40.6	39.9±0.5 (-3%)	38.3±0.5 (-7%)	38.0±0.5 (-8%)	45.1±0.5 (9%)	51.0±0.5 (24%)
Lactic acid	40.0	41.2±1.0 (3%)	42.5±1.0 (7%)	43.3±1.0 (8%)	53.9±1.1 (35%)	74.4±1.4 (86%)
Malic acid	39.8	42.0±1.8 (6%)	40.1±1.8 (1.3%)	40.7±1.8 (3%)	72.9±2.4 (84%)	77.6±2.6 (96%)

[a]RE (%) = $(C_f - C_t) / C_t * 100$

Table 2: Determination of AA in Vitamin C supplement.

Sample	Labeled concentration (mg per tablet)	Titrimetric method		Proposed method	
		Found value (mg per tablet)[a]	Recovery (%)	Found value (mg per tablet)[a]	Recovery (%)
Vitamin C supplement	500	522±18	105±4	521±9	104±2

[a]Data expressed as the mean ± SD, n=3.

Table 3: Determination of AA in natural orange juice sample.

Sample	Titrimetric method ($\mu g\ mL^{-1}$)[a]	Spiked value ($\mu g\ mL^{-1}$)	Proposed method	
			Found value ($\mu g\ mL^{-1}$)[a]	Recovery (%)
Natural orange juice	423±12	-	417±13	99±3
	423±12	16.5	433±13	98±4

[a]Data expressed as the mean ± SD, n=3.

Highlights:

- Vitamin C quantification is possible using a spectrometric smartphone-based system.
- Sensitivity enhancement is achieved by dispersive liquid-liquid microextraction.
- The microextraction procedure is optimized by multivariate optimization strategy.
- The analytical methodology is used in real-world samples.